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Research paper

Selective enzymatic degradation of poly(ε-caprolactone) containing multiblock copolymers

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Abstract

The hydrolytic and *Pseudomonas* lipase catalysed enzymatic degradation was studied for PDC multiblock copolymers consisting of poly(ε-caprolactone) (PCL) segments and poly(*p*-dioxanone) (PPDO) segments with variable composition. The enzymatic degradation of these multiblock copolymers is significantly accelerated by *Pseudomonas* lipase in contrast to the hydrolytic degradation where the degradation behaviour is determined by the PPDO segments. Degradation time intervals up to 200 h are selected, where the PPDO segments remain stable and do not contribute to the degradation process. A linear correlation between weight loss and increasing PCL content of the multiblock copolymers was found. X-ray diffraction data confirm that both crystalline and amorphous PCL are attacked by the enzymes. SEM cross-section images reveal that *Pseudomonas* lipase penetrates into the PDC polymers. The present study impressively demonstrates that selective enzymatic degradation of PCL containing multifunctional polymers is a beneficial tool for controlling their degradation properties.

Keywords: Enzymatic degradation; Hydrolytic degradation; Multiblock copolymer; Biodegradation; Shape-memory polymer; Segmented polyester urethanes

1. Introduction

Biodegradable synthetic polymers are widely applied in medical field, as for example in controlled drug release, in tissue engineering and as temporary implant materials [1–5]. For many applications there is a strong need for polymers with controlled degradation properties. A promising class of multifunctional biodegradable shape-memory polymers has been developed by Lendlein and co-workers [6–12]. These stimuli-sensitive materials have a high potential for applications as implants and surgical sutures for wound closure, especially in minimally invasive surgery. The degradation kinetics of shape-memory polymers is of enormous interest, as it is part of the multifunctional properties profile required for various medical applications. In the present degradation study multiblock copolymers, previously termed PDC, consist of poly(*p*-dioxanone) segments and poly(ε-caprolactone) switching segments, that are coupled via urethane bonds [12].

The hydrolytic degradation behaviour of poly(ε-caprolactone) and poly(*p*-dioxanone) homopolymers was studied in detail before by means of scanning electron microscopy, gravimetric measurements, nuclear magnetic resonance spectroscopy, differential scanning calorimetry and X-ray diffraction [13–19]. Macroscopic samples of both PCL and PPDO with dimensions up to several millimeter are described to undergo hydrolytic bulk degradation. The more hydrophilic poly(*p*-dioxanone) is hydrolysed much faster than the rather hydrophobic poly(ε-caprolactone). A weight loss of 25% within 10 weeks was observed in hydrolytic degradation experiments of PPDO samples,

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while PCL proved almost stable against hydrolytic attack for a time period of about nine months.

Besides numerous degradation studies concerning PCL and PPDO homopolymers, the degradation of PPDO–PCL copolymers was also investigated recently. Albuerne et al. described the hydrolytic degradation of PPDO-*b*-PCL diblock copolymers [20]. Hydrolytic degradation experiments of the PDC multiblock copolymers in aqueous buffer solution revealed a linear mass loss $\Delta m(t)$ that increased with increasing PPDO content [7]. A recent hydrolytic and enzymatic Langmuir monolayer degradation study of PDC multiblock copolymers disclosed a linear decrease of the Langmuir film area versus degradation time. Contrary to the hydrolytic degradation rate, the enzymatic degradation rate decreased with increasing content of PPDO blocks [21,22]. The degradation kinetics is explained based on a dangling chain model. Accordingly, the enzymatic scission of rather short PCL blocks in the PDC monolayers leads to sufficiently hydrophilic chain ends that become partly immersed in the degrading medium. Subsequently, these dangling chains get more efficiently hydrolysed than the chains constituting the Langmuir film.

A general problem related to biodegradable polymers is the fact that the *in-vitro* degradation data frequently do not comply with data of *in-vivo* degradation experiments [23].

Animal studies of PCL degradation revealed that the *in-vivo* degradation of PLC is significantly accelerated compared to the *in-vitro* experiments [24]. This is assigned to the optimum concentration of PCL-degrading enzymes, namely lipases, in the body. Further factors influencing the *in-vivo* degradation kinetics of polymers are mechanical stress, temperature, pH, the presence of ions and of species modifying the enzyme activity. The reduction of degradation experiments with laboratory animals should become possible based on *in-vitro* studies under conditions resembling those in a living organism. Thus, effective procedures for *in-vitro* studies of enzymatic degradation kinetics of biocompatible polymers are desirable.

The biodegradation of aliphatic polyesters in the presence of enzymes (esterase) and alkali is a topic of growing interest. Systematic *in-vitro* investigations dealing with the impact of enzymes on the degradation kinetics of synthetic polymers contribute to a better understanding of *in-vivo* degradation [25–42]. Several lipase type enzymes, as e.g. *Rhizopus delmer* lipase, *Rhizopus arrhizus* lipase, lipase *AK*, *Humicula lanuginosa* lipase and *Pseudomonas cepacia* lipase, were reported to significantly accelerate the degradation of poly(ε-caprolactone) [5,34–42].

The interfacial activation of lipase type enzymes mainly results from conformational changes in the enzymes. Approaching a substrate surface, they can expose their active site and provide a hydrophobic surface for interaction with the substrate chains. The enzyme conformation is strongly influenced by the solution conditions [39].

Grozev et al. analysed the hydrolysis of PCL homopolymers by *Humicola lanuginosa* lipase with the Langmuir monolayer degradation technique [40]. Wu et al. claimed that the presence of the microbial *Pseudomonas* lipase enhances the degradation rate of PCL nanoparticles by a factor of 1000 as compared with their purely hydrolytic degradation [41]. Li et al. [5] found biodegradation of PCL in PCL/PDLLA blends by *Pseudomonas* lipase to be significantly reduced compared to biodegradation of the pure PCL. He et al. analysed weight loss data of copolymers of γ-butyrolactone and ε-caprolactone degraded by *Pseudomonas* lipase [42]. They observed a decreasing degradation rate with increase of the γ-butyrolactone contents. Darwis et al. observed that an increasing cross-linking density reduces the enzymatic degradation rate of radiation cross-linked PCL samples [43].

The hydrolytic degradation of the multiblock copolymer PDC is reported to be mainly determined by the hydrolysis of the PPDO segments [7]. The motivation of the present work was to investigate whether the degradation kinetics of these polymers can be controlled by selective enzymatic degradation of the poly(ε-caprolactone) segment using *Pseudomonas* lipase. For this purpose the enzymatic degradation kinetics of PDC samples with different PPDO content and PPDO block length is investigated and compared with that of linear poly(ε-caprolactone) reference materials having different molecular weight.

2. Theoretical aspects

Several publications deal with the fundamentals of the theory of hydrolytic degradation and erosion of solid polymers [44–46]. The basic modes, the surface erosion and the bulk degradation, depend on the relation between the rate of water/enzyme diffusion into the polymer, the rate of chain cleavage by water ions/enzymes, and the rate of transportation of scission products out of the solid [44].

The rate of water diffusion into a polymer solid is strongly influenced by a number of structural parameters, as e.g. its porosity, the crystallinity, the surface roughness, the hydrophobicity and the size of the sample. While the penetration of enzymes in hydrogels is beyond dispute, most authors treat the enzymatic degradation of polymer solids exclusively as surface process. For hydrophilic enzymes it is usually considered to be difficult to penetrate into a hydrophobic polymer [17,39].

Surface- and bulk-segregation effects in blends and block copolymers can strongly influence the erosion kinetics of these materials by influencing the flexibility of chains [38], by enhancing the interface area of the homogeneous phases and by changing the diffusive transport through the system [47].

The comprehensive description of the hydrolytic and enzymatic degradation kinetics of polymers requires both a quantitative description of the chemical process of chain scission and a reliable model describing the physical transport phenomena of water and scission products involved.

The kinetics of hydrolytic chain scission of polyesters and the transient change of the molecular weight distribution can be described based on the theory of dynamic fragmentation [48]. The random hydrolytic degradation of polyesters causes the polymer chains to break down randomly via cascades of irreversible scission steps following a first order kinetic process. If the reaction rate constant k of hydrolysis is given, the theory of dynamic fragmentation can be applied to describe the influence of the initial molecular weight distribution of the degrading polymer on the generation rate of water-soluble degradation fragments. Thus, the degree of degradation of a polymer of given degradation rate constant k and molecular weight could be precisely derived, if the generation rate of soluble degradation fragments was known.

This was the motivation for hydrolytic degradation experiments with polymer Langmuir monolayers where the degradation kinetics are not influenced by transport processes like water or enzyme diffusion [21,22,40,49]. On the other hand, the additional parameters influencing the erosion of a real three-dimensional solid, as e.g. the penetration of water or enzymes into the solid and the transportation of eroded material out of the solid cannot be derived from monolayer experiments and require bulk-degradation data. The present bulk-degradation study of PDC multiblock copolymers and PCL reference materials aims to provide these bulk-degradation data for a comparison with the Langmuir degradation data of these materials published elsewhere [21,22].

3. Materials and methods

3.1. Materials

The investigated PDC multiblock copolymers were prepared from poly(ε-caprolactone)diol, $M_n = 2000\ \text{g mol}^{-1}$ purchased from Aldrich (Germany) and poly(*p*-dioxanone)diol $M_n = 1500\ \text{g mol}^{-1}$ coupled with 2,2(4),4-trimethyl-hexanediisocyanate as described by Lendlein et al. [10]. The PPDO contents of the synthesised polymers range from 33 wt% to 100 wt% according to the composition of the inserted telechelic oligomers. Besides the variation of the PPDO content in the multiblock copolymer the molecular weight of poly(*p*-dioxanone)diol was varied to $M_n = 5900\ \text{g mol}^{-1}$. These multiblock copolymers with shape-memory properties have been developed for medical applications and are biodegradable [6–12]. The transition temperature T_{trans} related to the thermally induced shape transition of PDC is the melting temperature of the semi-crystalline PCL switching segment, which is only slightly higher than body temperature to avoid any damage of surrounding tissue when heated to induce the shape-memory effect. The specification of the multiblock copolymers is given in Table 1 and in Fig. 1.

Linear poly(ε)-caprolactone with different molecular weight $M_n = 10{,}000\ \text{g mol}^{-1}$; $43{,}000\ \text{g mol}^{-1}$ and $80{,}000\ \text{g mol}^{-1}$ ($\text{C100}_{10\text{k}}$, $\text{C100}_{43\text{k}}$ and $\text{C100}_{80\text{k}}$) provided by Solvay Caprolactones (UK) was used as reference material without further purification.

Pseudomonas cepacia lipase is an enzyme promoting the cleavage of fatty acid esters. The lipase ($40\ \text{U mg}^{-1}$) isolated from soil bacteria *Pseudomonas* was purchased from Aldrich (Germany). The enzymes were dissolved in a potassium phosphate buffer solution (pH 7.2–7.6) purchased from Aldrich (Germany) to comply with *in-vitro* conditions. Hydrochloric acid (HCl) and sodium hydroxide (NaOH), used for pH-adjustment, were purchased from Merck (Germany).

3.2. Gel permeation chromatography (GPC)

The molecular weight of the multiblock copolymers was determined by gel permeation chromatography (GPC) using a Jasco system. It consists of a PU-1580 pump, of

Table 1
Composition and thermal properties of the PDC multiblock copolymers

Sample ID[a]	Polymer	Weight ratio PPDO/PCL (%)	T_{PCL}(DSC) (°C)	T_{PPDO}(DSC) (°C)
C00	[PPDO-(1.5*k*)-TMDI]$_n$	100/0	–	89.7
C67	PPDO-(1.5*k*)-TMDI-PCL(2*k*)	33/67	40.3	88.9
C50	PPDO-(1.5*k*)-TMDI-PCL(2*k*)	50/50	41.8	94.9
C33	PPDO-(1.5*k*)-TMDI-PCL(2*k*)	67/33	38.9	96.8
C50_H[b]	PPDO-(5.9*k*)-TMDI-PCL(2*k*)	50/50	40.0	90.4

The melting temperatures of the PCL segments T_{PCL} and of the PPDO segments T_{PPDO} are determined by DSC.

[a] C*xx*: *xx* is the weight percentage of PCL blocks.

[b] H stands for the higher molecular weight (5.9*k*) of the PPDO blocks.

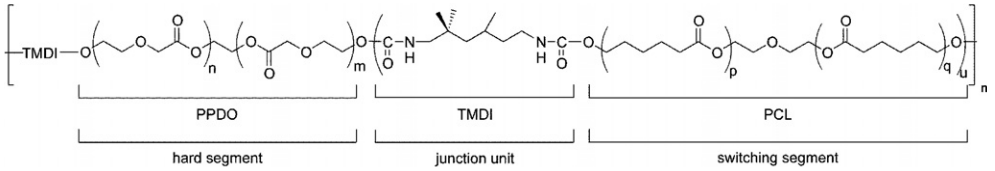

Fig. 1. Structural formula of the PDC multiblock copolymers.

300 mm × 8 mm linear PSS SDV-columns and of a UV- and a refractive index detector. Chloroform was used as eluent at a flow rate of $0.750\ \mathrm{ml\ min}^{-1}$ at room temperature.

3.3. Differential scanning calorimetry (DSC)

The thermal properties of the PDC multiblock copolymers were determined by differential scanning calorimetry (DSC). All DSC experiments were performed on a Netzsch DSC 204 Phoenix (Selb, Germany). All PDC samples were investigated in the temperature range from −100 °C to 150 °C with a constant heating and cooling rate of $10\ \mathrm{K\ min}^{-1}$. The samples were heated from 20 °C to 150 °C, then cooled down to −100 °C and again warmed up to 150 °C. All parameters were determined from the second heating run.

3.4. Enzymatic degradation procedure

For the enzymatic degradation studies the procedure as given in U.S. Pharmacopeia for simulated intestinal juice was followed [50]. Square size polymer film samples with physical dimensions of about 1 cm × 1 cm × 0.5 mm were cut. Each specimen was placed in a vial filled with 5 ml, pH 7.2 phosphate buffer (0.05 M) containing a well-defined amount of *P. cepacia* lipase. The vials were placed in water bath thermostated at 37 °C ± 0.2 °C. The buffer/enzyme system was changed every 24 h to restore the original level of enzymatic activity. Degradation studies were performed with samples immersed in the degrading medium for 24 h, 48 h, 72 h and 100 h. After immersion in the degrading medium, the specimens were removed, washed with distilled water (Milli-Q) and vacuum-dried at room temperature for one week before being subjected to analysis. For verification of the catalytic activity of enzymes versus the hydrolytic degradation the same procedure was followed using a pure aqueous buffer solution. Degradation induced morphological and structural changes were studied by gravimetric studies, X-ray diffraction (WAXS), scanning electron microscopy (SEM) and size exclusion chromatography (SEC, GPC). For gravimetric studies the samples were dried in a vacuum oven until weight constancy. The relative weight loss was plotted against the degradation time.

3.5. Wide angle X-ray scattering (WAXS)

The structure of the polymers was studied by wide angle X-ray diffraction (WAXD) in order to determine the relative amount of crystalline PCL- and PPDO-domains in the PDC multiblock copolymers, the evolution of the degree of crystallinity during degradation experiments and the changes in average crystal size. The nickel filtered radiation of a copper anode fine-focus tube (line focus) with a wavelength of 0.154 nm was used. The X-ray generator was operated at a voltage of 40 kV with a tube current of 30 mA. The primary beam was collimated by a slit system before hitting the sample mounted on the transmission sample holder of a two-circle goniometer HZG-4 (Seifert-FPM, Freiberg, Germany). The diffraction pattern was recorded by a proportional counter in the angular range between $2\theta = 0.5°$ and $2\theta = 35.5°$ using a step width of $\Delta(2\theta) = 0.05°$.

3.6. Scanning electron microscopy (SEM)

For scanning electron microscopy (SEM) investigations the dried samples were cut in small rectangular pieces and fixed on SEM sample holders. After gold–palladium coating within a cool sputter coater SCD 050 (Bal-tec, SEM Liechtenstein) the surface structure was studied in a high-resolution scanning electron microscope LEO 1550 (Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 3 kV under high vacuum conditions ($p \sim 10^{-6}$ Torr). For the cross-section experiments, the sample pieces were cut with a diamond knife of an ultra-microtome (UCT, Leica, Germany).

4. Results and discussion

The PDC multiblock copolymers C67, C50, $C50_H$, C33 and C00 composed of poly(ε-caprolactone) ($M_n = 2000\ \mathrm{g\ mol}^{-1}$) and poly(*p*-dioxanone) ($M_n = 1500\ \mathrm{g\ mol}^{-1}$ [for $C50_H$ $M_n = 5900\ \mathrm{g\ mol}^{-1}$]) with different segment weight ratio were studied with respect to their hydrolytic and enzymatic degradation properties in degradation media of variable enzyme concentration. The progress of the degradation process was analysed by means of gravimetric measurements, gel permeation chromatography, scanning electron microscopy and wide angle X-ray diffraction. For all investigated PDC multiblock copolymers the melting temperature of the poly(ε-caprolactone) segments was in the range of 39–42 °C (see Table 2). Thus, the PCL remained in a solid state during the degradation experiments performed at a temperature of 37 °C.

4.1. Enzymatic degradation behaviour of the PCL reference material

The PCL homopolymer samples, $C100_{10k}$, $C100_{43k}$ and $C100_{80k,}$ with different molecular weight were subjected to enzymatic and hydrolytic degradation, following the procedure described above. The enzymatic degradation kinetics was monitored by analysing the weight loss of the samples versus degradation time interval. For the reference material with the lowest molecular weight, $C100_{10k}$, the effect of the enzyme concentration on the bulk-degradation kinetics was studied in the range between $c_{\min} = 0$ (hydrolytic case) and $c_{\max} = 200\ \mu\mathrm{g\ ml}^{-1}$. The gravimetric results reveal no weight loss after 200 h immersion in the aqueous buffer solution. For the enzymatic degradation experiments a linear increase of the weight loss with the degradation time interval and with the enzyme concentration

Table 2
Changes in molecular weight of PDC multiblock copolymers during the enzymatic degradation

Sample ID[a]	Polymer	Weight ratio PPDO/PCL (%)	Molecular weight (GPC) degradation time 0 h		Molecular weight (GPC) degradation time 50 h		Molecular weight (GPC) degradation time 100 h	
			M_n (g mol^{-1})	PD	M_n (g mol^{-1})	PD	M_n (g mol^{-1})	PD
C00	[PPDO-(1.5*k*)-TMDI]$_n$	100/0	20,000	3.70	n.d.	n.d.	n.d.	n.d.
C67	PPDO-(1.5*k*)-TMDI-PCL(2*k*)	33/67	22,000	2.18	7000	4.93	n.d.	n.d.
C50	PPDO-(1.5*k*)-TMDI-PCL(2*k*)	50/50	19,000	2.10	5000	5.4	4000	5.25
C33	PPDO-(1.5*k*)-TMDI-PCL(2*k*)	67/33	14,000	2.21	8000	3.13	7000	3.08
C50$_H$[b]	PPDO-(5.9*k*)-TMDI-PCL(2*k*)	50/50	56,000	2.71	18,000	4.61	12,000	5.41

The molecular weights M_n are determined by GPC, PD is the polydispersity.

[a] C*xx*: *xx* is the weight percentage of PCL blocks.

[b] H stands for the higher molecular weight (5.9*k*) of the PPDO blocks.

was observed. The weight loss of C100$_{10k}$ bulk samples exposed for 72 h to lipase solutions of a concentration of 0.05 mg ml^{-1}, 0.1 mg ml^{-1} and 0.2 mg ml^{-1} amounts to (25 ± 3) wt%, (46 ± 4) wt% and (81 ± 6) wt%, respectively. This result confirms that the hydrolysis of PCL homopolymers is catalysed by *Pseudomonas* lipase and that the enzymatic degradation rate in the investigated concentration range is directly proportional to the enzyme concentration.

The impact of the initial molecular weight of PCL homopolymers on the enzymatic degradation kinetics is shown in Fig. 2. The variation of this parameter has a pronounced influence on the weight loss, which follows the predictions derived from the dynamic fragmentation theory [48], namely, the higher the initial molecular weight of the PCL homopolymers, the lower the weight loss of the respective sample. As only the shorter fragments of the PCL blocks become water-soluble and participate in the weight loss, longer sequences of this polymer require more scission steps to become degraded into sufficiently short fragments. This trend is nicely confirmed by monolayer degradation experiments [21,22]. In addition to this chemical aspect, the molecular weight of the polymers is expected to influence the diffusion phenomena, especially the penetration of water or enzymes into the polymer samples. As water diffusion into a semi-crystalline polymer is strongly influenced by the molecular weight, this physical aspect of polymer degradation requires further systematic studies. In near future, the continuously growing potential of molecular mechanics simulations of transport phenomena in polymers might improve our understanding of these physical phenomena involved in polymer degradation [51–53].

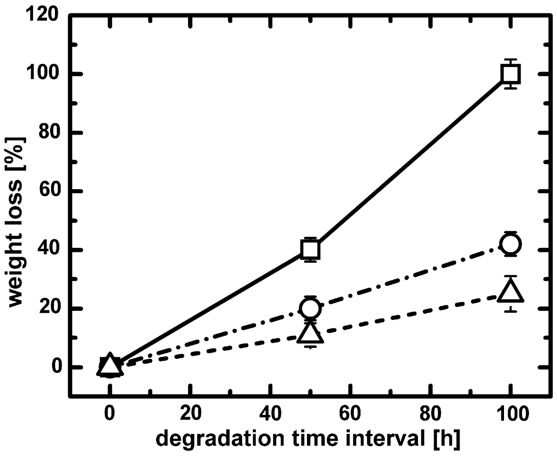

Fig. 2. Effect of molecular weight on enzymatic degradation of PCL homopolymers studied by gravimetric measurements: —□—: C100$_{10k}$; –·–○–·–: C100$_{43k}$; ----△----: C100$_{80k}$.

4.2. Enzymatic degradation behaviour of the PDC multiblock copolymers

The hydrolytic and enzymatic degradation experiments of the polymer C00 consisting of PPDO segments connected via TMDI linkers reveal an almost negligible initial weight loss of less than 4% within a degradation time interval of 200 h. This observation is in agreement with the findings of Sabino et al. [15] for PPDO homopolymers. Our experimental data confirm that on the one hand during degradation time intervals of up to 200 h there is no significant contribution of the PPDO segments to hydrolytic degradation of PDC samples and that on the other hand the degradation of PPDO is not catalysed by *Pseudomonas* lipase.

Fig. 3 shows the weight loss of different PDC multiblock copolymers with variable poly(ε-caprolactone) content during degradation time intervals of up to 100 h in a phosphate buffer (0.05 M) containing 200 μg ml^{-1}of enzyme. A linear correlation between weight loss rate and PCL content in the polymers is found. For the sample C67 with the highest PCL content the highest weight loss is observed. This material lost about 75% of weight after 100 h enzymatic degradation and its physical structure collapsed entirely. The weight of the samples decreases almost linearly with the immersion time in the enzyme solution, leading to an almost constant weight loss rate.

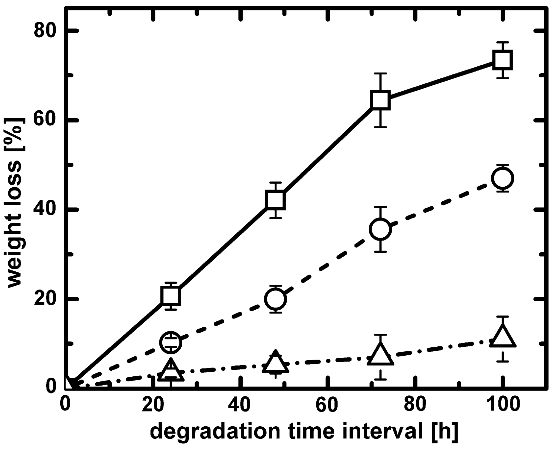

Fig. 3. Effect of PPDO:PCL segment weight ratio in PDC multiblock copolymers on the degradation behaviour by an aqueous buffer solution containing 200 μg ml^{-1} *Pseudomonas* lipase derived from gravimetric measurements: —□—: C67; ----○----: C50; –·–△–·–: C33.

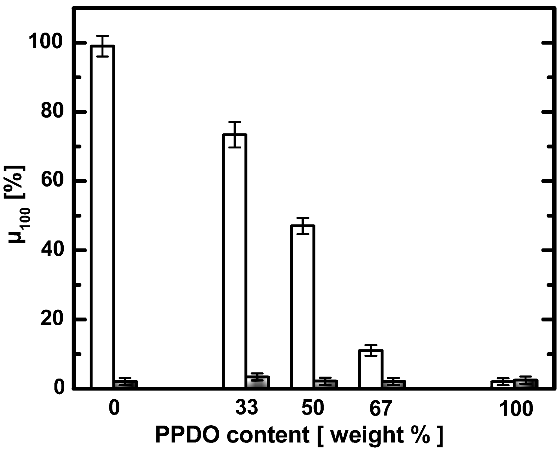

Fig. 4. Relative weight loss μ of PDC samples at 37 °C as function of the PPDO content: black bars after 100 h immersion in aqueous buffer solution (pH 7.2) and white bars after 100 h immersion in buffered (pH 7.2) *Pseudomonas* lipase solution (200 μg ml^{-1}).

The molecular weights M_n of the PDC multiblock copolymers determined by GPC after enzymatic degradation time intervals of 0 h, 50 h and 100 h are listed in Table 2. As a result of the enzymatic degradation a significant decrease in molecular weight M_n in combination with an increase of the polydispersity was found for all PDC multiblock copoymers.

The highest weight loss rate and a pronounced increase in polydispersity combined with a significant reduction in molecular weight are observed for the PDC multiblock copolymer having the highest PCL content (sample C67), while a lower weight loss and a lower increase in polydispersity combined with a slight reduction in molecular weight are observed for the PDC multiblock copolymer having the highest PPDO content (sample C33). Samples C50 and C50$_{\mathrm{H}}$ exhibit the most pronounced decrease in molecular weight combined with a maximum increase in polydispersity after 100 h enzymatic degradation while showing an intermediate weight loss rate.

In Fig. 4 the hydrolytic degradation data from gravimetric studies of PDC- and PCL- samples are compared with enzymatic degradation data. The values for the sample with 0% PPDO are derived from experiments with PCL homopolymers with $M_n = 10{,}000$ g mol^{-1}, while the PCL diols used as educt in the PDC multiblock copolymer synthesis have $M_{n(\mathrm{PCL})} = 2000$ g mol^{-1}. Due to the enormous influence of the polymer molecular weight on the chain scission kinetics (compare Fig. 2) the gravimetric data of the PCL sample must not be directly compared with those of the PDC samples, as the different degradation kinetic is most probably caused by these different initial molecular weights. After 100 h enzymatic degradation the weight loss for all polymers was considerably higher than that observed in hydrolytic degradation experiments. The apparent acceleration of degradation by the presence of *Pseudomonas* lipase is directly proportional to the weight percentage of PCL blocks in the PDC multiblock copolymers. During *in-vitro* enzymatic degradation of C67 the solution turned turbid and the overall weight loss of these samples was higher than the total PCL content in the polymer. These observations suggest a loss of PPDO fragments. The maximum weight loss after 100 h immersion of samples C50 and C33 in the enzyme solution did not reach values corresponding to the total PCL content in multiblock copolymers. The weight loss rate of these samples is linearly correlated with the enzyme concentration, as already observed for the PCL homopolymers.

Scanning electron microscopy (SEM) images of the dried polymer samples provided a deeper insight into the morphological changes induced by the degradation process. The SEM images of C67 samples degraded for 24 h and 72 h in buffer solution and in lipase solution (0.2 mg ml^{-1}), respectively, are compared in Fig. 5. No changes of surface roughness were found after immersion in the buffer solution for up to 72 h. After 24 h enzymatic degradation, however, the film surface got strongly roughened and grainy structures appeared at the surface, and after 72 h the film roughness further increased due to the co-existence of materials with different stability against enzyme attack.

The most probable explanation of these observations is the preferred enzymatic degradation of the amorphous poly(ε-caprolactone), while PCL crystallites and PPDO segments withstand the enzyme attack for longer time. Finally, grains of PPDO and PCL crystallites get released and become immersed in the buffer solution, turning it turbid.

The SEM surface images of samples C50 and C33 taken after 100 h enzymatic degradation do not show such pronounced changes in surface roughness (see Fig. 6). Cross-section images of samples C50$_{\mathrm{H}}$ and C33 after 100 h enzymatic degradation reveal morphological changes in the upper surface region of the samples (Fig. 7) attributed to enzyme penetration. The thickness of this surface layer

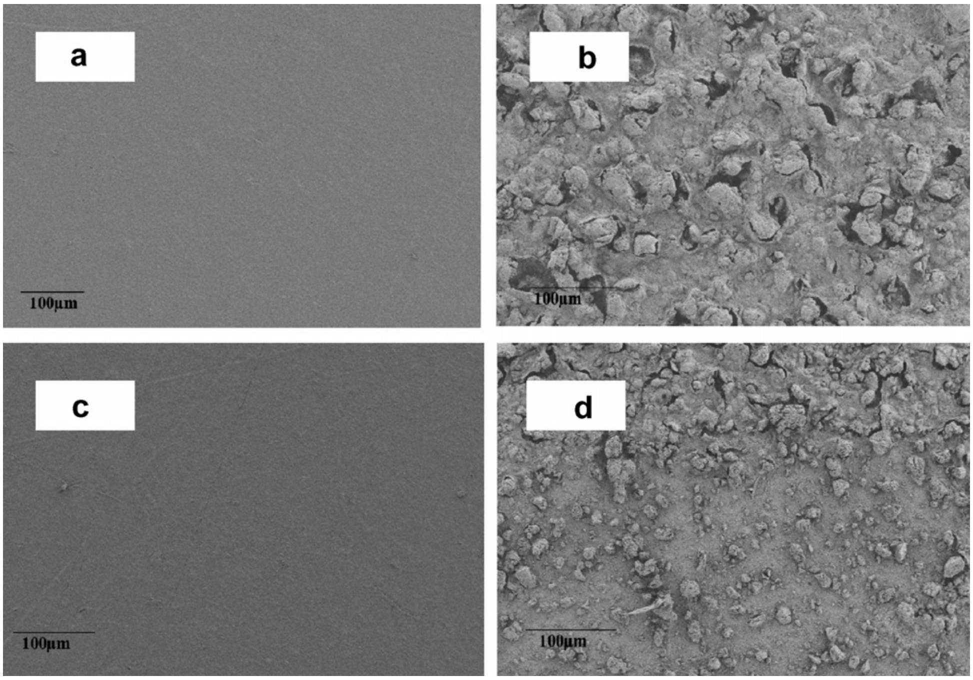

Fig. 5. SEM topography images of degraded C67 samples after 24 h (a) and 72 h (c) exposure to aqueous phosphate buffer solution (pH 7.2, $T = 37$ °C) and to buffered (pH 7.2) *Pseudomonas* lipase solution (200 µg ml^{-1}, $T = 37$ °C), respectively, (b) and (d).

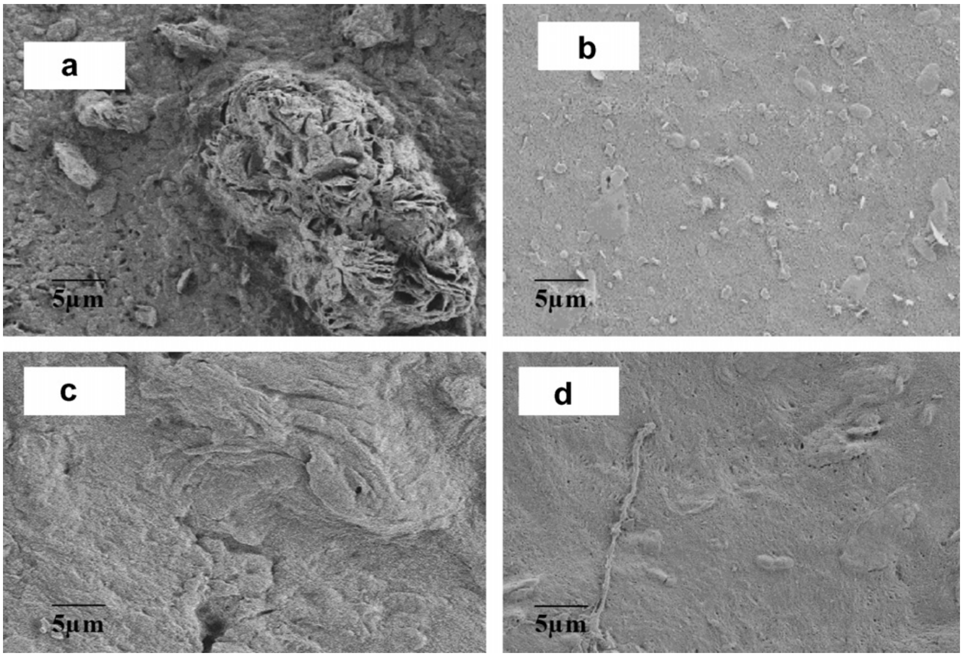

Fig. 6. SEM images of partially degraded PPDO–PCL multiblock copolymers after 100 h exposure to phosphate buffered (pH 7.2) *Pseudomonas* lipase solution (200 µg ml^{-1}, $T = 37$ °C): (a) C67; (b) C33; (c) C50; and (d) C50$_H$.

increases almost linearly with enzymatic degradation time, as exemplified for two of the materials in Fig. 8.

The comparison of the apparent enzyme penetration depth of samples C50$_H$ and C33 indicates the penetration velocity of enzymes to be influenced by the PCL content and/or the molecular weight of the PPDO blocks. The resulting apparent depth of enzyme penetration at a given enzyme concentration is a linear function of the degradation time. The cross-section images clearly prove that enzymes penetrate into the bulk of the PDC multiblock copolymers and modify the volume structure in the vicinity of the sample surface, while the material deep inside the sample is not effected. Vacancies remaining after local degradation of PCL segments by the lipase are considered to

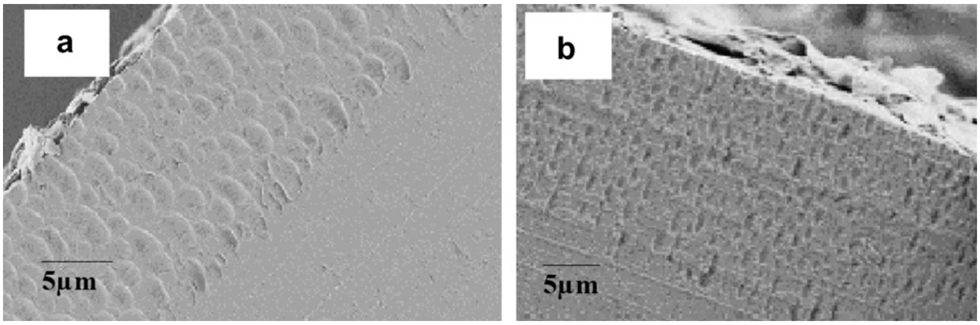

Fig. 7. SEM cross-section images of samples after 100 h exposure to aqueous phosphate buffer solution (pH 7.2, $T = 37$ °C) containing 200 μg ml^{-1} *Pseudomonas* lipase showing that this lipase penetrates into the bulk of PDC multiblock copolymers: (a) C50$_H$ and (b) C33.

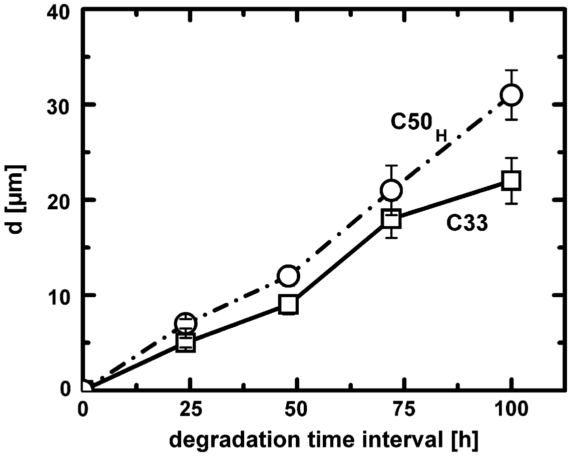

Fig. 8. Apparent penetration depth *d* of *Pseudomonas* lipase (200 μg ml^{-1}) into the bulk of PDC multiblock copolymers derived from SEM cross-section images. –·–○–·–: C50$_H$ and —□—: C33.

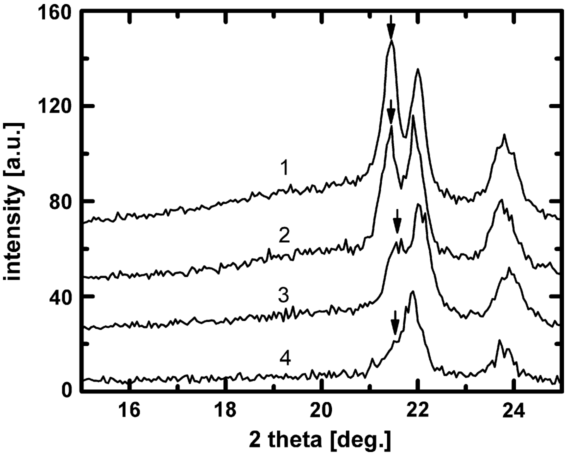

Fig. 10. Wide angle X-ray diffraction pattern of C67 before (1) and after 24 h (2), 48 h (3) and 72 h (4) exposure to aqueous phosphate buffer solution (pH 7.2, $T = 37$ °C) containing 200 μg ml^{-1} *Pseudomonas* lipase.

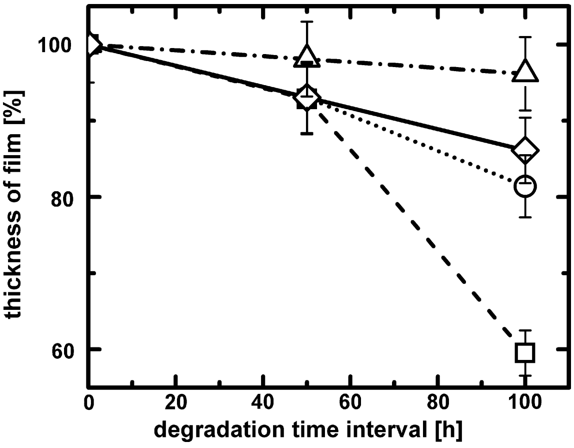

Fig. 9. Reduction in film thickness studied for PPDO–PCL multiblock copolymers over 100 h exposure to aqueous phosphate buffer solution (pH 7.2, $T = 37$ °C) containing 200 μg ml^{-1} *Pseudomonas* lipase: ----□----: C67; ····○····: C50; –·–△–·–: C33; —◇—: C100$_{80k}$.

be the penetration path of further enzymes into the bulk of the PDC multiblock copolymers. The true penetration depth of enzymes can, however, not be derived directly from cross-section images, as during enzymatic degradation the upper surface of the samples is moving toward the centre of the sample due to surface erosion. Thus, the apparent penetration depth does not include the way of enzymes through the meanwhile completely eroded outmost surface layer. The approximate extension of this layer can be derived from measurements of the overall sample thickness. The reduction of sample thickness after immersion in the enzyme solution and drying may result either from erosion of the uppermost layer or from shrinking of the degraded, porous surface regions. Fig. 9 shows the reduction in total film thickness as function of the enzymatic degradation time interval. The highest reduction in overall film thickness is observed in sample C67. The thickness reduction values of the PDC multiblock copolymer samples are directly proportional to the PCL content in the copolymers.

The wide angle X-ray scattering (WAXS) technique provides insight into the changes of the physical structure of the PDC multiblock copolymers during enzymatic degradation. Fig. 10 displays the wide angle X-ray scattering (WAXS) patterns of C67 before enzymatic degradation and after multiples of 24 h of enzymatic degradation time intervals. The WAXS patterns show a decrease in amorphous scattering intensity with increasing degradation

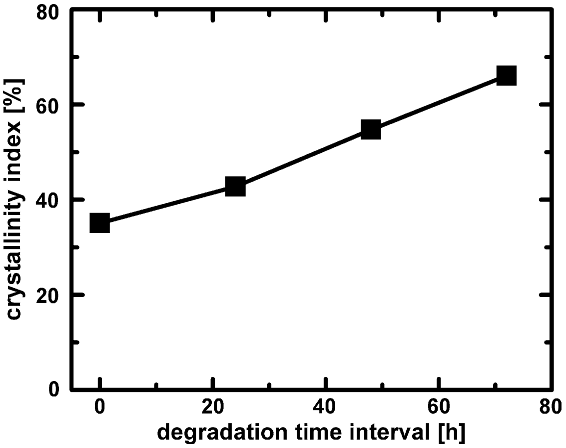

Fig. 11. Influence of exposure to aqueous phosphate buffer solution (pH 7.2, $T = 37\,^{\circ}C$) containing 200 µg ml^{-1} *Pseudomonas* lipase on the crystallinity index calculated from WAXS data of sample C67.

time, indicating the preferred enzymatic degradation of amorphous poly(ε-caprolactone) segments. The integrated peak intensity ratio of the isolated PCL-(110)-peak and the adjacent peak originating from both PPDO- and PCL-crystals decreases with increasing degradation time. The relative crystalline PCL content C_{PCL} is derived from integrated peak intensity ratio of the crystalline PCL-(110)-peak and adjacent mixed PPDO–PCL peak. For polymer C67 the relative crystalline PCL content decreases from $C_{\mathrm{PCL}} = 67\%$ for the virgin sample to $C_{\mathrm{PCL}} = 35\%$ after 72 h enzymatic degradation. This observation confirms that enzymes do also degrade crystalline PCL, which is in agreement with data published by Gan et al., who studied degradation of crystalline PCL in poly(ε-caprolactone)/poly(DL-lactide) blends by lipase [36,37]. The total crystalline X-ray scattering intensity I_{C} of the degrading sample C67 gradually decreases up to about 48 h degradation time, but after 72 h of enzymatic degradation a sudden loss in absolute crystalline scattering I_{C} of the samples is observed. Simultaneously, the total amorphous scattering I_{A} of all our PDC multiblock copolymers decreases linearly with degradation time. The fact, that the enzymatic degradation of amorphous material proceeds faster than that of crystalline parts, is reflected by the overall increase of the crystallinity index C, calculated from the ratio between I_{C} and the total X-ray scattering ($I_{\mathrm{C}} + I_{\mathrm{A}}$) (see Fig. 11).

5. Conclusions

The hydrolytic and *Pseudomonas* lipase catalysed enzymatic degradation was studied for series of PDC multiblock copolymer samples consisting of slowly hydrolysing poly(ε-caprolactone) segments and fast hydrolysing poly(*p*-dioxanone) segments with variable PCL content and of PCL homopolymers with different molecular weight.

For the enzymatic degradation experiments with PCL homopolymers a linear increase of the weight loss with both the degradation time and the enzyme concentration was observed. The PCL degrades the faster, the lower its initial molecular weight. For poly(ε-caprolactone) with a molecular weight of $M_n = 10{,}000$ g mol^{-1} a weight loss of more than 80% was observed after 72 h enzymatic degradation.

The enzymatic degradation of PDC multiblock copolymers is strongly accelerated by *Pseudomonas* lipase with respect to the hydrolytic case. A linear correlation between weight loss and increasing PCL content in the PDC was found. No significant hydrolytic or enzymatic cleavage of the PPDO segments was observed within the degradation time intervals of up to 200 h. Further we found that *Pseudomonas* lipase penetrates the PDC multiblock copolymers. The velocity of the enzyme penetration front moving into a planar PDC film sample can be derived from SEM cross-section images taken at different degradation time intervals, considering the overall reduction of the total film thickness. The velocity of enzyme penetration seemed to be influenced by the PCL content and/or the molecular weight of the PDC multiblock copolymers. A more detailed study of enzyme penetration is necessary to separate the influence of the different structure parameters on the penetration kinetics. The enzymatic degradation of PDC multiblock copolymers by Pseudomonas lipase is accompanied by an almost linear weight loss, as already found for the purely hydrolytic degradation of these materials. The linear kinetics observed in case of the enzymatic Langmuir monolayer degradation of PDC multiblock copolymers was recently described by a dangling chain model based on a random chain scission process [22]. This model is obviously restricted to the outmost layer of a bulk sample, but the underlying random chain scission of the short PCL blocks is supposed to apply to the PDC bulk samples, as well. The linear enzymatic bulk-degradation kinetics observed here is best explained by a surface erosion mechanism of the PDC samples. After reaching steady state conditions both enzyme penetration front and temporary sample surface move into the bulk. The influence of the observed broadening of the degradation zone between these planes on the degradation rate may be partly compensated by the delayed transport of degradation products out of this zone, leading to the observed linear kinetics.

The results of this degradation study show that selective enzymatic degradation is a beneficial tool for controlling the degradation properties of multifunctional polymers, as e.g. block copolymers consisting of blocks with different sensitivity against enzymatic attack. Both the opportunity of tuning the degradation kinetics of biodegradable polymers and the structures resulting from selective enzymatic degradation of segregated block copolymers can lead to new attractive applications in the field of implant materials, scaffolds and drug release systems.

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